Research Article

Pharmacophore Mapping and Molecular Docking Studies on Coffin-Lowry Syndrome using Insilico Techniques

P. Sahithi[1]*, A. Ravindernath[2], P. Raja Rao[3], Kamineni Reddy Bhargavi[4]
[1]*Assistant Professor, University College of Technology, Osmania University, Hyderabad, India.
[2]Professor and Head, University College of Technology, Osmania University, Hyderabad, India.
[3]Associate Professor, University College of Technology, Osmania University, Hyderabad, India.
[4]M.Tech Biotechnology Final year, University College of Technology, Osmania University, Hyderabad, India.
*Corresponding author's E-mail: sahithi.c@gmail.com

ABSTRACT

The 90 kDa ribosomal S6 kinases (RSKs) are a family of serine/threonine kinases activated by extracellular signal-regulated protein kinase (ERK1 and ERK2) in response to many growth factors, peptide hormones and neurotransmitters. RSK2, one of the isoform of RSK protein contains two kinase domains connected by a regulatory linker sequence. The C-terminal kinase domain (CTK) belongs to the CamK family and is activated by ERK-type MAP kinases, leading to auto-phosphorylation of RSK2 at Ser386 in a hydrophobic motif while the N-terminal kinase domain (NTK) belongs to the AGC kinase family and is activated by 3-phosphoinositide dependent protein kinase-1 (PDK1) through phosphorylation of Ser227, and is responsible for phosphorylation of substrates of RSK protein. Coffin-Lowry syndrome (CLS), a rare disorder characterized by mental retardation and dysmorphisms is associated with genetic defects in the RSK2 gene leading to inactivation of RSK2 and hampering its functions. Pharmacophore mapping and docking studies were performed and new ligands were identified to activate PDK1 which in turn activates RSK2 by phosphorylation. Further, activated RSK2 has been proposed to phosphorylate the substrates like CREB and histone H3, after translocation to the nucleus which is controlled by the protein called PEA-15 (a small death effector domain-containing protein). Protein-protein docking studies between PEA-15 and RSK2 have been performed in order to study how PEA-15 regulates the RSK2-mediated signaling.

Keywords: Coffin-Lowry syndrome (CLS), Docking, Dysmorphism, Neurotransmitters, Pharmacophore, Ribosomal S6 Kinase (RSK).

INTRODUCTION

Genetics is the study of genes, heredity, and genetic variation in living organisms. It is generally considered a field of biology, but it intersects frequently with many of the life sciences and is strongly linked with the study of information systems. A syndrome is a collection of recognizable traits or abnormalities that tend to occur together and are associated with a specific disease. A genetic syndrome is an illness caused by one or more abnormalities in the genome, especially a condition that is present from birth. Most genetic syndromes are quite rare and affect one person in every several thousands or millions.

Coffin-Lowry syndrome (CLS) is an X-linked disorder characterized by psychomotor retardation, hypotonia and characteristic facial, hand and skeletal malformations. Typically male patients are of short stature and exhibit a characteristic coarse face with a prominent forehead, microcephaly, hypertelorism, downslanting palpebral fissures, ptosis, prominent supraorbital ridges, epicanthic folds, thick lips, a thick nose septum with anteverted nares, and irregular or missing teeth. The estimated incidence of Coffin-Lowry syndrome is 1 in 50,000 to 1 in 100,000, and about 70 to 80% of patients are sporadic cases.

RSKs[1] are serine-threonine protein kinases, acting in the Ras-Mitogen-Activated Protein Kinase (MAPK)[3,4] signalling pathway. RSKs are directly phosphorylated and activated by ERK1 and 2 (Extracellular Signal-Regulated Kinases) in response to a broad range of cellular perturbations, including stimulation with insulin and growth factors, neurotransmitters, oncogenic transformation and UV-irradiation.

Homology model of the DHRS7B protein created with Swiss-model and rendered with PyMOL. Evolutionarily related proteins have similar sequences and naturally occurring homologous proteins have similar protein structure. It has been shown that three-dimensional protein structure is evolutionarily more conserved than would be expected on the basis of sequence conservation alone.

MATERIALS AND METHODS

There are hundreds of genomics databases: some are comprehensive, but are not carefully curated (GenBank), while others are carefully curated, but are narrow (FlyBase).

The Protein Data Bank (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. (See also crystallographic database.)

The data, typically obtained by X-ray crystallography[5] or NMR spectroscopy and submitted by biologists and biochemists from around the world.

Input: Input sequences are in FASTA or Genbank format and weight matrix.

Output: BLAST output can be delivered in a variety of formats. These formats include HTML, plain text, and XML formatting. For NCBI's web-page, the default format for output is HTML. When performing a BLAST on NCBI, the results are given in a graphical format showing the hits found, a table showing sequence identifiers for the hits with scoring related data, as well as alignments for the sequence of interest and the hits received with corresponding BLAST scores for these.

If one is attempting to search for a proprietary sequence or simply one that is unavailable in databases available to the general public through sources such as NCBI, there is a BLAST program available for download to any computer, at no cost. This can be found at BLAST+ executables. There are also commercial programs available for purchase. Databases can be found from the NCBI site, as well as from Index of BLAST databases. BLAST performs sequence alignment through following steps. The first step is to create a list of all possible words from query sequence called seeding. Each word is typically three residues for protein and eleven residues for nucleotide sequences. The second step is to search a sequence database for the occurrence of these words. Third step is to match the words, and is scored by a given substitution matrix. A word is considered as a match if it's above the threshold. The fourth step involves pairwise alignment by extending from the words in both the directions while counting the alignment score using same substitution matrix, and continues until the score of the alignment drops below the threshold due to mismatches (the drop threshold is twenty-two for proteins and twenty for DNA). The resulting contiguous aligned segment pair without gaps is called high-scoring segment pair. In the original the version of BLAST the high scored HSPs are presented as the final report. They are also called as maximum scoring pairs.
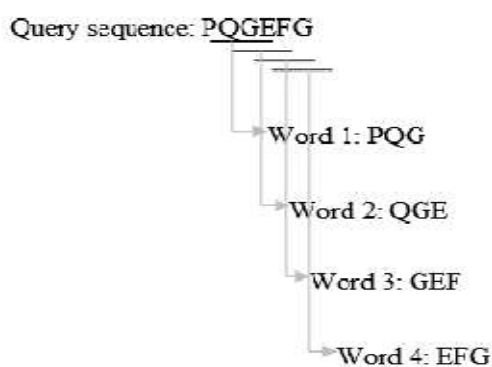

Figure 1: The method to establish query word list

Draw and view structures in 2D, or render in 3D to view from any angle. Draw reactions and reaction schemes, and calculate reactant quantities. Generate structures from InChI and SMILES strings. Generate IUPAC systematic names for molecules of up to 50 atoms and 3 ring structures. Predict logP for individual structures. Search for structures in the built-in dictionary of over 165,000 systematic, trivial, and trade names.

Interaction Generation protocol in the DS was used in generating the pharmacophore from the active site of the protein. This protocol extracts pharmacophore query from the Ludi interaction map which is created inside the active site sphere[2] and only assigns three main features namely HBA, HBD and HY. All the parameters within this protocol were left as their default values. To the generated pharmacophore, ligand pharmacophore mapping protocol was employed that maps and aligns to the pharmacophore. Based on the fit value which is a measure of how well the ligand fits the pharmacophore, the ligand with highest fit value was selected.
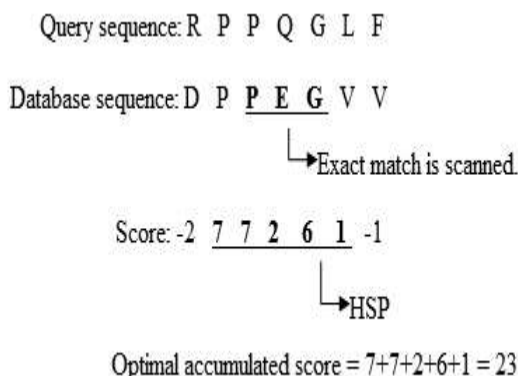

Figure 2: The process to extend the exact match

RESULTS AND DISCUSSION

In the present study target protein i.e., RSK2, which is playing a major role in the study of the syndrome is tested for its activity with various ligand compounds.

Sequence Retrieval and Template Selection

The amino acid sequence of RSK2 was retrieved in FASTA format from SWISSPROT database with accession number P51812 and submitted to BLAST against PDB database for the selection of template for homology modeling. From the BLAST results, based on identity, score and E-value, the template with highest identity was selected.

Primary sequence of RSK2 is taken from Protparam

Number of amino acids: 740

Figure 3: Amino acid sequence of RSK2 protein

Sequence Alignment and Model Building

Alignment of the sequence of the template-target pair is an essential input in homology modeling and is carried

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out using Clustal W. "MODELLER 9.14v". The refinement process was carried out using DS by applying CHARMm force field. Steepest descent method with 0.001 minimizing RMS gradient and 2000 minimizing steps followed by conjugant gradient method was applied till satisfactory results obtained for minimization.

3D-structure prediction done by homology modeling method.

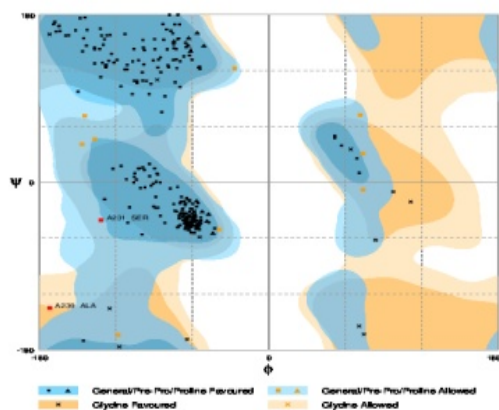PDK1Selection of protein structure

1H1W

Resolution: 2.00

Aa: 71-359

To evaluate the quality of the modelled structure of RSK2, the final refined model was analyzed by the RAMPAGE Server, and RMSD calculation.

Ramachandran Plot Analysis

RAMPAGE Server was used for validation of protein structure which revealed that the modeled target structure has significant stereo-chemical quality in Ramachandran plot with favorable region (96.0%), allowed region (3.2%), and disallowed region (0.7%) respectively.
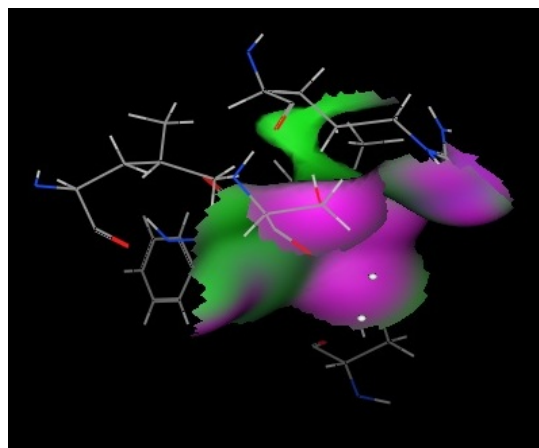

Figure 4: Ramchandran plot analysis of RSK2 protein

Figure 5: Active site pocket identification using site finder from MOE

Docking studies Results

Table 1: Results from docking studies with respective ligands

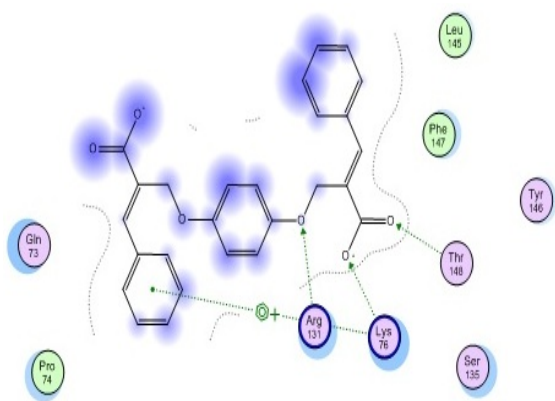| S. No.	Ligand	Wild type	Mutant-1 (Arg 131 mutated to Ala 131)	Mutant-2 (Lys 76 mutated to ALa 76)	Mutant-3 (Thy 148 mutated to Ala 148)
1.	S-1	-12.1897	-11.0589	-7.4050	-10.0561
2.	S-2	-11.5748	-10.9983	-8.6427	-10.7908
3.	S-3	-11.4891	-11.9650	-8.5722	-11.1123

Figure 6: Interaction of the protein with the ligand S-4

Table 2: Interaction of both Protein 1,A and Protein 2,E using Protein-Protein Docking

Protein 1, A	Protein 2, E
His 54	Asp 58
Arg 192	Lys 122
Glu 58	Arg83
Asp 250	Lys 122

CONCLUSION

RSK2 appears to be required for correct neuronal development since Coffin-Lowry individuals frequently exhibit severe psychomotor retardation. Literature survey revealed that CLS results due to inactivation of RSK2. In

the present study, pharmacophore mapping and docking studies were performed in order to design new molecular entities that would activate RSK2 in CLS individuals. Further, in order to study how PEA-15 controls activated RSK2-mediated signaling, protein-protein docking studies were performed between the two proteins.

Out of 740 amino acids that were considered in the present study, 14 amino acids were having potential active docking sites, which were analyzed using Ramachandran plot analysis. 7 ligands were prepared insilico using discovery studio, which were docked with the protein using Modeller 9.14.

The ligand S-5 (2E)-2-[(napthalen-2-yloxy) methyl]-3-phenylprop-2-enoic acid, which has shown a fitness value of 1.996 by using pharmacophore mapping. Therefore, without the use of any wet lab techniques, online tools and servers have played a crucial role in docking and pharmacophore mapping studies of Coffin-Lowry Syndrome. There is a wide scope for further research using these *in silico* designing in other prevalent genetic syndromes.

REFERENCES

1. Alcorta D.A., Crews C.M., Sweet L.J., Bankston L., Jones S.W., Erikson R.L. *Mol. Cell Biol.*, 9(9), 1989, 3850-3859.

2. Biondi R.M., Cheung P.C., Casamayor A., Deak M., Currie R.A. and Alessi D.R. Identification of a pocket in the PDK1 kinase domain that interacts with PIF and the C-terminal residues of PKA.EMBO J., 19, 2000, 979±988.

3. Chong H., H. G. Vikis, and K. L. Guan. Mechanisms of regulating the Raf kinase family. Cell Signal. 15, 2003, 463–469.

4. Deak M., A. D. Clifton, L. M. Lucocq, and D. R. Alessi. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK andSAPK2/p38, and may mediate activation of CREB. EMBO J. 17, 1998, 4426–4441.

5. Johnson L.N. and Lewis R.J. Structural basis for control by phosphorylation. Chem. Rev., 101, 2001, 2209±2242.

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